Molecules and Mechanisms

After 28 years, environmental health scientists at the NIEHS Environmental Health Sciences Center at Oregon State University (OSU) are still trendsetters. The research agenda has matured with the times to a focus on the mechanisms by which toxic substances affect living tissues. Today researchers observe events that were barely discernible in the center's infancy, coupling the latest mass spectrometry techniques with cell culture and DNA gene splicing to peer deep into subcellular workings. "In molecular biology, and particularly in nucleic acid sequencing, something that had taken you two months to do in '65 now takes a day," points out center Director Donald Reed, a distinguished professor of biochemistry. "You can ask questions at a level we would've once thought impossible."

Early Years

For almost as long as chemical companies have promoted pesticides as the means to gain miraculous increases in crop yields, researchers at OSU have carefully investigated the unexpected side effects and unintended distribution of these chemicals. The concern began well before World War II, when arsenic and other inorganic chemicals were the main members of the farmer's arsenal. After the deluge of synthetic pesticides in the mid-1940s, scientists from OSU's agricultural chemistry department began investigating incidents like fish kills in the wake of improper use of pesticides, the appearance of chlorinated hydrocarbon and organic phosphorous-containing insecticides in fish and soil sediments, and pesticide residues in fruits and vegetables.

By 1964, concerned researchers at the university had amassed sufficient evidence of the problems with pesticides to receive an NIH program grant to study them. The program, "Toxicology of Pesticides in the Environment," ultimately led to OSU earning one of the first Environmental Health Sciences Center research core grants from the National Cancer Institute in 1967. When the NIEHS was founded in 1969, it took over these grants. The current research program grant, "Toxicology of Environmental Chemicals," funds areas of research such as the biochemical and molecular mechanisms through which exposure to dioxins affects T-lymphocytes and how this is related to specific kinds of immunosuppression. It also supports research in which scientists expose *E. coli* bacteria to ultraviolet light and sulfur dioxide and look for mutations to investigate how UV radiation and sulfur dioxide, which associated with respiratory disease, cause damage to DNA. Humans are exposed to sulfur dioxide through automobile exhaust and food and wine additives.

Under Reed's leadership since 1981, the center has shifted its focus toward molecular biology and toxicology, making it one of the first centers to focus on mechanisms of action. The center's researchers were recently reorganized into groups reflecting the center's major areas of inquiry. These research core units include carcinogenesis, cell biology and immunotoxicology, molecular and genetic toxicology, and structural and environmental chemistry.

Two Centers in One Campus

Across from OSU's Environmental Health Sciences Center in Corvallis is the university's Marine/Freshwater Biomedical Sciences Center. One of the five specialty centers funded by the NIEHS, the MFBS center's close proximity allows for a rich mix of information and faculty. In addition to directing the MFBS Center, George Bailey, distinguished professor of food toxicology, is also the co-leader of the main environmental health sciences center's carcinogenesis research core. (The OSU center also has ties to the Oregon Health Sciences University in Portland which includes a medical school.)

Even before the adjunct center for studies of aquatic environmental issues was formed, the OSU center was respected for its interdisciplinary pursuits. When the center received its first university funding in 1966, Virgil Freed, director of the Department of Agricultural Chemistry, solicited help from scientists in the departments of psychology, fisheries and wildlife, engineering, nuclear chemistry, veterinary medicine, and agricultural economics. This collection of researchers from varied disciplines was indisputably effective in helping achieve some of the center's major research breakthroughs.

For example, researchers at the center generated much of the data that ultimately resulted in DDT being banned in the United States and have led the field in dioxin toxicity investigations. Center research into the physical basis for widespread distribution of man-made chemicals into the environment helped explain how pesticides made their way into the polar ice caps. Researchers at the OSU center conducted the earliest investigations into the mechanisms behind the preferential effects of pesticides on certain species and have made major contributions to understanding how protective systems aid in coping with exposure to toxicants. Research at the center has also contributed to the understanding of the mechanisms of action of aflatoxin, a widely recognized food carcinogen, the heart's muscarinic receptors, changes in which are associated with Alzheimer's disease, cardiac arrhythmias, and pesticide poisoning, and the cytochrome P450s affected by disruption of enzyme regulation.

Extending Expertise

The OSU center consists of an integrated staff of researchers from departments such as agricultural chemistry, biochemistry and biophysics, food science and technology, statistics, veterinary medicine, and zoology, whose expertise complements each other.

For example, the specialized knowledge of mass spectrometry represented by the structural and environmental chemistry research core has helped Dale Mosbaugh, a professor of agricultural chemistry and the leader of the molecular and genetic toxicology research core investigating DNA repair,

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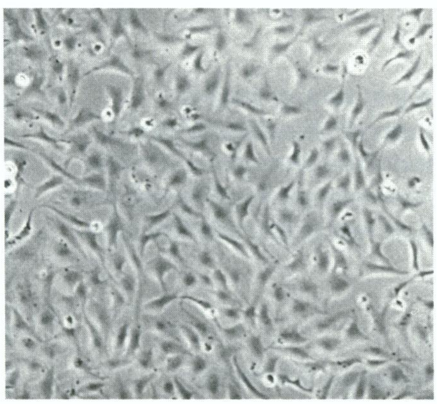
Partners in progress (left to right): George Bailey, director of the MFB Center; Ken Olden, director of NIEHS; Anne Sassaman, director of extramural research and training, NIEHS; and Donald Reed, director of the OSU EHS Center.

perform analyses. Recalling a project that attempted to define the domains of the uracil DNA glycosylase protein that interacted and bound to DNA, Mosbaugh said, "Our approach was to cross-link the enzyme to the DNA and isolate for the cross-linked peptide fragments. The peptides would define what parts of the protein were actually contacting the DNA. We ran into a bit of a roadblock, in that we had difficulty resolving all of the cross-linked peptides because the DNA that we'd cross-linked it to dominated their properties." Mosbaugh learned that another center researcher, Doug Barofsky, professor of agricultural chemistry and leader of the structural and environmental chemistry research core, was cross-linking proteins with DNA, then analyzing by mass spectrometry the size of those cross-linked products. Because of this research, Barofsky was able to suggest a different approach. Said Mosbaugh, "I'm not sure I would've known [the answer] as soon had we not both been in the center . . . Working at the center means I'm able to interact with other members and feel that their expertise and equipment are an extension of my own."

A physicist by training, Barofsky built the center's two matrix-assisted desorption-ionization time-of-flight (MALDI-TOF) spectrometers used to conduct such research. In collaboration with Mosbaugh, he recently confirmed the formation of a uracil DNA glycosylase–DNA complex by using ultraviolet light to permanently bind the two together and then using MALDI-TOF mass spectometry to identify the peptide sequences defining the DNA binding domain. This was the first time this technique had been used to establish contact points in a DNA–protein complex. Barofsky is now focusing on the process by which DNA and proteins interact.



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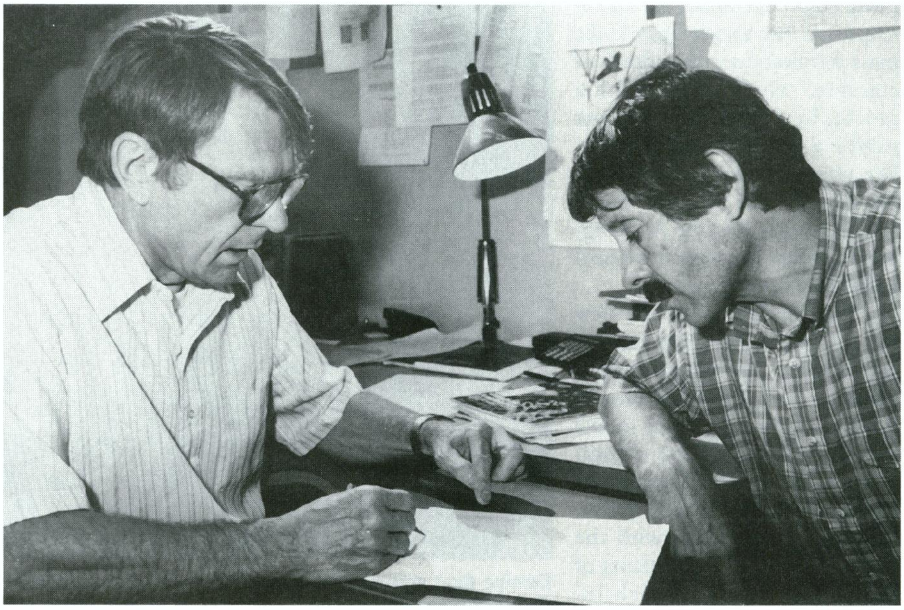
Culture club. Center researchers use zebrafish embryo cell cultures to examine Ah receptor-mediated responses to toxicants.



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Experts in mass spec. Max Deinzer (left) and Douglas Barofsky discuss mass spectrometric approaches to center research.

Collaborations of this type have allowed the center to excel in DNA repair research. Building on previous research investigating the enzymatic nature involved in uracil–DNA repair, Mosbaugh is now involved in elucidating the biochemical and biological role of the uracil–DNA repair pathway in human fibroblasts.

Max Deinzer, professor of agricultural chemistry and deputy director of the center, and Michael Schimerlik, professor of biochemistry and biophysics and also a deputy director of the center, are using an electrospray-ionization mass spectrometer to investigate the folding kinetics of proteins, specifically the human recombinant macrophage colony-stimulating factor, by measuring the ratios of deuterium to hydrogen. "We're trying to determine how the proteins form and what environmental chemicals will do to upset the formation," Deinzer explained. Deinzer, Schimerlik, and Mosbaugh also hope to use the technique to observe how the uracil *N*-glycosylase inhibitor protein folds.

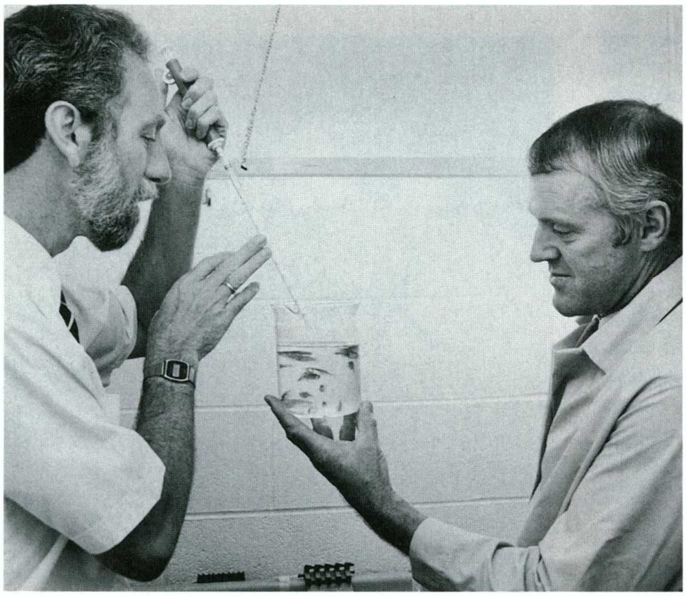
The cell biology and immunotoxicology research core is led by David Barnes, a professor of biochemistry and biophysics. A decade ago Barnes used an unorthodox approach to create a medium on which neural stem cells could grow normally without senescence; previously, biologists believed that cells could not continue to grow normally for an indefinite period. Currently Barnes is developing *in vitro* culture techniques suitable for introducing and expressing exogenous DNA in zebrafish and trout cells to examine them for aryl hydrocarbon (Ah) receptor-mediated responses.

Nonmammalian Alternatives

Barnes's research is one example of a major focus of the OSU center on developing alternative models for research into chemical carcinogenesis. Another is the rainbow trout model, which established the value of nonmammalian alternatives for carcinogenesis studies. Trout, like humans, are resistant to peroxisome proliferation. Because peroxisome proliferation has been shown to cause liver cancer in rodents, but not in humans, the trout model allows scientists to explore these mechanisms. "Fish are incredibly economical in terms of space requirements and rearing constraints," explained Bailey. "We can focus attention on issues in cancer dose response that are really statistically quite challenging. Therefore, we are able to propose and carry out studies with from 10,000 to 30,000 animals at a fraction of the cost for rodents. We can establish a relationship between a carcinogen and/or an anticarcinogen and the amount of damage, then statistically quantify the relationship in a way not possible with rodent models."

A model currently under investigation by the cell biology and immunotoxicology research core uses zebrafish. Zebrafish are advantageous as a model because they are available year round and reach sexual maturity in three months. They are especially suitable for embryonic studies, Barnes points out, because fertilization occurs outside the body. "Besides being observable under the microscope, it's easy to synchronize the mass fertilization of hundreds of embryos," says Barnes. "With a mouse, by comparison, you get maybe a dozen if you're lucky. We ultimately have our eyes on the dioxin [Ah] receptor. Some studies suggest that the Ah receptor has a key function in normal embryonic development."

The possibilities of the new fish model are especially exciting considering the breakthroughs achieved with the trout model. Bailey has used the trout model to study the mechanisms of inhibition of chemical carcinogenesis for more than a dozen years. Building on successes with the indole-3-carbinol from plants of the genus *Brassica,* which include broccoli, cauliflower, and cabbages and have shown promise against human breast cancer, Bailey recently researched the chemoprotective properties of chlorophyllin, a form of the chlorophyll found in all green plants. The studies were the first to prove in an animal model that chlorophyllin could inhibit experimental cancer, primarily by trapping carcinogens in the intestinal tract. Eighty percent tumor inhibition was achieved with only 1–2% of chlorophyll levels found in common spinach. Other scientists are following up with rodent studies and human trials are under consideration at The Johns Hopkins University.



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Earning their stripes. Fast-maturing zebrafish are used by center scientists George Bailey (left) and Jerry Hendricks as animal models in cell biology and immunotoxicology research.

Outreach

In addition to sharing their expertise among themselves, the center's scientists have long recognized the need to share their findings with researchers outside the center. The center's strong ties to the College of Agricultural Sciences at OSU and the state's Cooperative Extension service at OSU have greatly aided in this effort. Researcher Nancy Kerkvliet, professor of agricultural chemistry, now directs the outreach program. "Our mission is to bring the latest in university research to the public," said Kerkvliet. "We focus on presenting the relative risks of pesticides in general, as well as environmental chemicals like domoic acid [a shellfish contaminant] and dioxins."

At present, the center's focus remains man-made chemicals, with the exception of Bailey's research into chemoprotective materials. In the future the center may also investigate how naturally occurring substances fit into the toxicology picture. "As we understand more and more about the mechanisms of man-made chemicals, we wonder, are there things that are naturally existing that do the same thing in the same way?" said Reed. "We know that tobacco and alcohol make some impact in terms of outcomes of whatever environmental exposures we have. We need to do more to demonstrate the actual role of these components so that people have a better basis for making decisions about their individual lifestyles."

Kellyn Betts